PALM INTRANET

Day : Friday
Date: 9/26/2003

Time: 09:06:04

Inventor Name Search

Enter the **first few letters** of the Inventor's Last Name.
Additionally, enter the **first few letters** of the Inventor's First name.

Last Name	**First Name**	
engelman	alan	Search

To go back use Back button on your browser toolbar.

Back to PALM | ASSIGNMENT | OASIS | Home page

PALM INTRANET

Day : Friday
Date: 9/26/2003

Time: 09:06:04

Inventor Name Search

Enter the **first few letters** of the Inventor's Last Name.
Additionally, enter the **first few letters** of the Inventor's First name.

Last Name	First Name	
hofmann	wolfgang	Search

To go back use Back button on your browser toolbar.

Back to PALM | ASSIGNMENT | OASIS | Home page

HILIGHT set on as ''
HILIGHT set on as ''
? begin 5,6,55,154,155,156,312,399,biotech,biosci
>>>   135 is unauthorized

```
      Set  Items  Description
      ---  -----  -----------
? s (retrovir? or lentivir?) (5n) episom? and integrase
          557070  RETROVIR?
          117935  LENTIVIR?
           62205  EPISOM?
             194  (RETROVIR? OR LENTIVIR?)(5N)EPISOM?
           17641  INTEGRASE
      S1       0  (RETROVIR? OR LENTIVIR?) (5N) EPISOM? AND INTEGRASE
? s (retrovir? or lentivir?) (10n) (episom? or extrachromosom?) and integrase
          557070  RETROVIR?
          117935  LENTIVIR?
           62205  EPISOM?
           23055  EXTRACHROMOSOM?
             369  (RETROVIR? OR LENTIVIR?)(10N)(EPISOM? OR EXTRACHROMOSOM?)
           17641  INTEGRASE
      S2      16  (RETROVIR? OR LENTIVIR?) (10N) (EPISOM? OR
                  EXTRACHROMOSOM?) AND INTEGRASE
? rd s2
...completed examining records
      S3       7  RD S2 (unique items)
? d s3/3/1-7
      Display 3/3/1     (Item 1 from file: 5)
DIALOG(R)File   5:Biosis Previews(R)
(c) 2003 BIOSIS. All rts. reserv.

11810907   BIOSIS NO.: 199900057016
Mutations in nonconserved domains of Ty3 integrase affect multiple
  stages of the Ty3 life cycle.
AUTHOR: Nymark-Mcmahon M Henrietta; Sandmeyer Suzanne B(a)
AUTHOR ADDRESS: (a)Dep. Biol. Chem., Univ. California Irvine, 240D Med.
  Sci. I, Irvine, CA 92697-1700**USA
JOURNAL: Journal of Virology  73 (1):p453-465 Jan., 1999
ISSN: 0022-538X
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

                                - end of record -
?
      Display 3/3/2     (Item 1 from file: 154)
DIALOG(R)File 154:MEDLINE(R)
(c) format only 2003 The Dialog Corp. All rts. reserv.

10049157   21988564   PMID: 11991751
  A novel, helper-dependent, adenovirus-retrovirus hybrid vector: stable
transduction by a two-stage mechanism.
  Soifer Harris; Higo Collin; Logg Christopher R; Jih Lily Ja-Lu;
Shichinohe Toshiaki; Harboe-Schmidt Erik; Mitani Kohnosuke; Kasahara
Noriyuki
  Institute for Genetic Medicine, University of Southern California, 2250
Alcazar Street, CSC-240, Los Angeles, California 90033, USA.
  Molecular therapy - the journal of the American Society of Gene Therapy (
United States)  May 2002,  5  (5 Pt 1)  p599-608,  ISSN 1525-0016
Journal Code: 100890581
  Contract/Grant No.: R21DK54280; DK; NIDDK
  Document type: Journal Article
  Languages: ENGLISH
  Main Citation Owner: NLM
  Record type: Completed

                                - end of record -
```

?
Display 3/3/3 (Item 1 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)

124334831 CA: 124(25)334831w PATENT
Method of using transdominant negative retroviral integrase in the treatment of retroviral infection
INVENTOR(AUTHOR): Holler, Tod Paul; Meyer, Annette; Nabel, Gary Jan; Post, Leonard
LOCATION: USA
ASSIGNEE: Warner-Lambert Company
PATENT: PCT International ; WO 9604386 A1 DATE: 960215
APPLICATION: WO 95US9110 (950719) *US 286578 (940805)
PAGES: 44 pp. CODEN: PIXXD2 LANGUAGE: English CLASS: C12N-015/48A; C12N-015/55B; A61K-048/00B; C12N-015/86B; C12N-005/10B
DESIGNATED COUNTRIES: CA; EE; JP; LT; LV; MX; SI DESIGNATED REGIONAL: AT ; BE; CH; DE; DK; ES; FR; GB; GR; IE; IT; LU; MC; NL; PT; SE

- end of record -

?
Display 3/3/4 (Item 1 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

06094163 Genuine Article#: XU704 No. References: 48
Title: New insight on the role of **extrachromosomal retroviral** DNA
Author(s): Cara A (REPRINT) ; Reitz MS
Corporate Source: MT SINAI MED CTR,DIV INFECT DIS, 1 GUSTAVE LEVY PL, BOX 1090, ATRAN 6-620/NEW YORK//NY/10029 (REPRINT); NCI,BASIC RES LABS, NIH/BETHESDA//MD/20892; UNIV MARYLAND,INST BIOTECHNOL, INST HUMAN VIROL/BALTIMORE//MD/21201; SCH MED,/BALTIMORE//MD/21201
Journal: LEUKEMIA, 1997, V11, N9 (SEP), P1395-1399
ISSN: 0887-6924 Publication date: 19970900
Publisher: STOCKTON PRESS, HOUNDMILLS, BASINGSTOKE, HAMPSHIRE, ENGLAND RG21 6XS
Language: English Document Type: REVIEW (ABSTRACT AVAILABLE)

- end of record -

?
Display 3/3/5 (Item 1 from file: 98)
DIALOG(R)File 98:General Sci Abs/Full-Text

04751949 H.W. WILSON RECORD NUMBER: BGSA02001949 (USE FORMAT 7 FOR FULLTEXT)
Mechanisms of retroviral recombination.
Negroni, Matteo
Buc, Henri
Annual Review of Genetics v. 35 (2001) p. 275-302
SPECIAL FEATURES: bibl il ISSN: 0066-4197
LANGUAGE: English
COUNTRY OF PUBLICATION: United States
WORD COUNT: 12929

- end of record -

?
Display 3/3/6 (Item 2 from file: 98)
DIALOG(R)File 98:General Sci Abs/Full-Text

04274002 H.W. WILSON RECORD NUMBER: BGSA00024002 (USE FORMAT 7 FOR

FULLTEXT)
Pathogenicity islands and the evolution of microbes.
Hacker, Jorg
Kaper, James B
Annual Review of Microbiology v. 54 (2000) p. 641-79
SPECIAL FEATURES: bibl diag tab ISSN: 0066-4227
 LANGUAGE: English
COUNTRY OF PUBLICATION: United States
WORD COUNT: 17927

- end of record -

?
      Display 3/3/7   (Item 3 from file: 98)
DIALOG(R)File 98:General Sci Abs/Full-Text
(c) 2003 The HW Wilson Co. All rts. reserv.

04255640   H.W. WILSON RECORD NUMBER: BGSA00005640   (USE FORMAT 7 FOR
FULLTEXT)
Plant retrotransposons.
AUGMENTED TITLE: review
Kumar, Amar
Bennetzen, Jeffrey L
Annual Review of Genetics v. 33 (1999) p. 479-532
SPECIAL FEATURES: bibl il ISSN: 0066-4197
 LANGUAGE: English
COUNTRY OF PUBLICATION: United States
WORD COUNT: 22407

- end of display -
? s integrase (5n) (mutat? or modif? or defectiv?)
Processing
Processed 10 of 34 files ...
Completed processing all files
          17641 INTEGRASE
        2149184 MUTAT?
        3811373 MODIF?
         311101 DEFECTIV?
      S4   1019 INTEGRASE (5N) (MUTAT? OR MODIF? OR DEFECTIV?)
? s s4 and (retrovir? or lentivir?) (3n) vector?
           1019 S4
         557070 RETROVIR?
         117935 LENTIVIR?
        1218379 VECTOR?
          53417 (RETROVIR? OR LENTIVIR?)(3N)VECTOR?
      S5   49 S4 AND (RETROVIR? OR LENTIVIR?) (3N) VECTOR?
? rd s5
...completed examining records
      S6   17 RD S5 (unique items)
? d s6/3/1-17
      Display 6/3/1   (Item 1 from file: 5)
DIALOG(R)File   5:Biosis Previews(R)
(c) 2003 BIOSIS. All rts. reserv.

13796575   BIOSIS NO.: 200200425396
Integrase-LexA fusion proteins incorporated into human immunodeficiency
  virus type 1 that contains a catalytically inactive integrase gene are
  functional to mediate integration.
AUTHOR: Holmes-Son Michelle L; Chow Samson A(a)
AUTHOR ADDRESS: (a)Department of Molecular and Medical Pharmacology, UCLA
  AIDS Institute, and Molecular Biology Institute, UCLA School of Medicine,
  Los Angeles, CA, 90095**USA E-Mail: schow@mednet.ucla.edu
JOURNAL: Journal of Virology 74 (24):p11548-11556 December, 2000
MEDIUM: print

ISSN: 0022-538X
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

- end of record -

?
Display 6/3/2 (Item 2 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

12830783 BIOSIS NO.: 200100037932
High-level expression of active HIV-1 integrase from a synthetic gene in human cells.
AUTHOR: Cherepanov Peter; Pluymers Wim; Claeys Anje; Proost Paul; De Clercq Erik; Debyser Zeger(a)
AUTHOR ADDRESS: (a)Rega Institute for Medical Research, K.U. Leuven, Minderbroedersstraat 10, B-3000, Leuven: zeger.debyser@uz.kuleuven.ac.be **Belgium
JOURNAL: FASEB Journal 14 (10):p1389-1399 July, 2000
MEDIUM: print
ISSN: 0892-6638
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English
SUMMARY LANGUAGE: English

- end of record -

?
Display 6/3/3 (Item 3 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

11966388 BIOSIS NO.: 199900219701
Stable transduction of quiescent CD34+CD38- human hematopoietic cells by HIV-1-based **lentiviral vectors**.
AUTHOR: Case Scott S; Price Mary A; Jordan Craig T; Yu Xiao Jin; Wang Lijun ; Bauer Gerhard; Haas Dennis L; Xu Dakun; Stripecke Renata; Naldini Luigi ; Kohn Donald B; Crooks Gay M(a)
AUTHOR ADDRESS: (a)Childrens Hospital Los Angeles, 4650 Sunset Blvd., MS No. 62, Los Angeles, CA, 90027**USA
JOURNAL: Proceedings of the National Academy of Sciences of the United States of America 96 (6):p2988-2993 March 16, 1999
ISSN: 0027-8424
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English
SUMMARY LANGUAGE: English

- end of record -

? d s6/9/3
Display 6/9/3 (Item 3 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

11966388 BIOSIS NO.: 199900219701
Stable transduction of quiescent CD34+CD38- human hematopoietic cells by HIV-1-based **lentiviral vectors**.
AUTHOR: Case Scott S; Price Mary A; Jordan Craig T; Yu Xiao Jin; Wang Lijun ; Bauer Gerhard; Haas Dennis L; Xu Dakun; Stripecke Renata; Naldini Luigi ; Kohn Donald B; Crooks Gay M(a)
AUTHOR ADDRESS: (a)Childrens Hospital Los Angeles, 4650 Sunset Blvd., MS No. 62, Los Angeles, CA, 90027**USA

JOURNAL: Proceedings of the National Academy of Sciences of the United States of America 96 (6):p2988-2993 March 16, 1999
ISSN: 0027-8424
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English
SUMMARY LANGUAGE: English

-more-

?
Display 6/9/3 (Item 3 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

ABSTRACT: We compared the efficiency of transduction by an HIV-1-based **lentiviral vector** to that by a Moloney murine leukemia virus (MLV) **retroviral vector**, using stringent in vitro assays of primitive, quiescent human hematopoietic progenitor cells. Each construct contained the enhanced green fluorescent protein (GFP) as a reporter gene. The **lentiviral vector**, but not the MLV vector, expressed GFP in nondivided CD34+ cells (45.5% GFP+) and in CD34+CD38- cells in G0 (12.4% GFP+), 48 hr after transduction. However, GFP could also be detected short-term in CD34+ cells transduced with a **lentiviral vector** that contained a **mutated integrase** gene. The level of stable transduction from integrated vector was determined after extended long-term bone marrow culture. Both MLV **vectors** and **lentiviral vectors** efficiently transduced cytokine-stimulated CD34+ cells. The MLV vector did not transduce more primitive, quiescent CD34+CD38- cells (n = 8). In contrast, stable transduction of CD34+CD38- cells by the **lentiviral vector** was seen for over 15 weeks of extended long-term culture

-more-

? d s6/9/4-17
Display 6/9/4 (Item 4 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

11119937 BIOSIS NO.: 199799741082
Highly efficient and sustained gene transfer in adult neurons with a **lentivirus vector.**
AUTHOR: Blomer Ulrike; Naldini Luigi; Kafri Tal; Trono Didier; Verma Inder M; Gage Fred H(a)
AUTHOR ADDRESS: (a)Lab. Genetics, Salk Inst. Biol. Studies, PO Box 85800, San Diego, CA 92186-5800**USA
JOURNAL: Journal of Virology 71 (9):p6641-6649 1997
ISSN: 0022-538X
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The identification of monogenic and complex genes responsible for neurological disorders requires new approaches for delivering therapeutic protein genes to significant numbers of cells in the central nervous system. A **lentivirus**-based **vector** capable of infecting

-more-

?
Display 6/9/4 (Item 4 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

dividing and quiescent cells was investigated in vivo by injecting highly concentrated viral vector stock into the striatum and hippocampus of adult rats. Control brains were injected with a Moloney murine leukemia virus, adenovirus, or adeno-associated virus vector. The volumes of the

areas containing transduced cells and the transduced-cell densities were stereologically determined to provide a basis for comparison among different viral vectors and variants of the viral vector stocks. The efficiency of infection by the **lentivirus vector** was improved by deoxynucleoside triphosphate pretreatment of the vector and was reduced following **mutation** of **integrase** and the Vpr-matrix protein complex involved in the nuclear translocation of the preintegration complex. The **lentivirus vector** system was able to efficiently and stably infect quiescent cells in the primary injection site with transgene expression for over 6 months. Triple labeling showed that 88.7% of striatal cells transduced by the **lentivirus vector** were terminally differentiated neurons.

-more-

?
Display 6/9/4 (Item 4 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

DESCRIPTORS:
MAJOR CONCEPTS: Cell Biology; Genetics; Methods and Techniques; Microbiology; Nervous System (Neural Coordination)
BIOSYSTEMATIC NAMES: Adenoviridae--Viruses; Muridae--Rodentia, Mammalia, Vertebrata, Chordata, Animalia; Parvoviridae--Viruses; Retroviridae--Viruses
ORGANISMS: adeno-associated virus (Parvoviridae); adenovirus (Adenoviridae); lentivirus (Retroviridae); rat (Muridae); Moloney murine leukemia virus (Retroviridae)
BIOSYSTEMATIC CLASSIFICATION (SUPER TAXA): animals; chordates; mammals; microorganisms; nonhuman mammals; nonhuman vertebrates; rodents; vertebrates; viruses
MISCELLANEOUS TERMS: Research Article; ADULT; GENE VECTOR; GENETIC METHOD; METHODOLOGY; MOLECULAR GENETICS; NERVOUS SYSTEM; NEURON; STRIATAL CELL; TERMINALLY DIFFERENTIATED; VIRUS-MEDIATED GENE TRANSFER
CONCEPT CODES:
02506 Cytology and Cytochemistry-Animal

-more-

?
Display 6/9/4 (Item 4 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

03506 Genetics and Cytogenetics-Animal
10052 Biochemical Methods-Nucleic Acids, Purines and Pyrimidines
20501 Nervous System-General; Methods
31500 Genetics of Bacteria and Viruses
33506 Virology-Animal Host Viruses
BIOSYSTEMATIC CODES:
02601 Adenoviridae (1993-)
02618 Parvoviridae (1993-)
02623 Retroviridae (1993-)
86375 Muridae

- end of record -

?
Display 6/9/5 (Item 1 from file: 154)
DIALOG(R)File 154:MEDLINE(R)

10012937 21945803 PMID: 11945062
Correct integration mediated by integrase-LexA fusion proteins incorporated into HIV-1.
Holmes-Son Michelle L; Chow Samson A
Department of Molecular and Medical Pharmacology, UCLA AIDS Institute,

Los Angeles, California, 90095, USA.
Molecular therapy - the journal of the American Society of Gene Therapy (United States) Apr 2002, 5 (4) p360-70, ISSN 1525-0016
Journal Code: 100890581
Contract/Grant No.: CA68859; CA; NCI
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS
Fusion of wild-type or truncated integrase to a sequence-specific

-more-

?
Display 6/9/5 (Item 1 from file: 154)
DIALOG(R)File 154:MEDLINE(R)

DNA-binding protein, such as the Escherichia coli LexA repressor, results in an integration bias toward the recognition site of the DNA-binding protein in vitro. **Integrase-defective** HIV-1 could become integration-competent by supplying the fusion protein in trans. To understand the mechanism of complementation, the virus-host DNA junctions of cells infected with the integrase-LexA containing virus were sequenced. The characteristic hallmarks of wild-type integration were present, a 5'-TG/CA-3' at the ends of the viral sequence and a 5-bp direct repeat in the immediately flanking cellular DNA. Experiments were also carried out to determine the mechanism by which the amino- or carboxy-terminal truncated integrase fused to LexA restored integration to the integrase-mutant viral clone. Complementation experiments using purified fusion proteins in vitro, or viruses encoding a C-terminal truncated integrase and containing various fusion proteins in trans, indicated that the truncated integrase-LexA proteins are inactive per se and they restore integration by forming mixed multimers with the virally encoded mutant integrase. Correct integration of retroviral DNA by the in trans method illustrates the feasibility of

-more-

?
Display 6/9/5 (Item 1 from file: 154)
DIALOG(R)File 154:MEDLINE(R)

introducing integrase fusion proteins into **retroviral vectors** to achieve site-directed integration without interfering with the attributes of the integration reaction.
Tags: Human; Support, Non-U.S. Gov't; Support, U.S. Gov't, P.H.S.
Descriptors: *Bacterial Proteins--metabolism--ME; *HIV-1--genetics--GE; *Integrase--metabolism--ME; *Serine Endopeptidases--metabolism--ME; *Virus Integration; Amino Acid Substitution; Bacterial Proteins--genetics--GE; Chimeric Proteins--genetics--GE; Chimeric Proteins--metabolism--ME; Codon, Nonsense; Escherichia coli Proteins--genetics--GE; Escherichia coli Proteins--metabolism--ME; Genetic Complementation Test; Genetic Vectors --genetics--GE; HIV-1--enzymology--EN; HIV-1--metabolism--ME; Hela Cells; **Integrase**--genetics--GE; **Mutation**; Proviruses--genetics--GE; Serine Endopeptidases--genetics--GE; Virion--metabolism--ME
CAS Registry No.: 0 (Bacterial Proteins); 0 (Chimeric Proteins); 0 (Codon, Nonsense); 0 (Escherichia coli Proteins); 0 (Genetic Vectors); 0 (lexA protein)
Enzyme No.: EC 2.7.7.- (Integrase); EC 3.4.21 (Serine Endopeptidases)

-more-

?
Display 6/9/5 (Item 1 from file: 154)
DIALOG(R)File 154:MEDLINE(R)

Record Date Created: 20020411
Record Date Completed: 20020927

- end of record -

?
Display 6/9/6 (Item 2 from file: 154)
DIALOG(R)File 154:MEDLINE(R)
(c) format only 2003 The Dialog Corp. All rts. reserv.

09617559 21403274 PMID: 11511376
Self-excising **retroviral vectors** encoding the Cre recombinase overcome Cre-mediated cellular toxicity.
Silver D P; Livingston D M
The Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA 02115, USA.
Molecular cell (United States) Jul 2001, 8 (1) p233-43, ISSN 1097-2765 Journal Code: 9802571
Contract/Grant No.: K08CA82572; CA; NCI
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS
The Cre-lox system is often used to manipulate sequences in mammalian genomes. We have observed that continuous expression of the Cre recombinase

-more-

?
Display 6/9/6 (Item 2 from file: 154)
DIALOG(R)File 154:MEDLINE(R)
(c) format only 2003 The Dialog Corp. All rts. reserv.
in cultured cells lacking exogenous lox sites caused decreased growth, cytopathic effects, and chromosomal aberrations. Cre mutants defective in DNA cleavage were not geno- or cytotoxic. A self-excising **retroviral vector** that incorporates a negative feedback loop to limit the duration and intensity of Cre expression avoided measurable toxicity, retained the ability to excise a target sequence flanked by lox sites, and may provide the basis of a less toxic strategy for the use of Cre or similar recombinases.
Tags: Animal; Human; Support, U.S. Gov't, P.H.S.
Descriptors: DNA--metabolism--ME; *Genetic **Vectors**; *Integrase --metabolism--ME; ***Retroviridae**--genetics--GE; *Transfection--methods --MT; *Viral Proteins--metabolism--ME; 3T3 Cells; Cell Division; Cell Line; Chromosome Aberrations; Genes, Reporter; Integrase--genetics--GE; **Integrase**--toxicity--TO; Mice; **Mutation**; Recombinant Fusion Proteins--metabolism--ME; Retroviridae--metabolism--ME; Viral Proteins --genetics--GE; Viral Proteins--toxicity--TO
CAS Registry No.: 0 (Genetic Vectors); 0 (Recombinant Fusion Proteins)

-more-

?
Display 6/9/6 (Item 2 from file: 154)
DIALOG(R)File 154:MEDLINE(R)
(c) format only 2003 The Dialog Corp. All rts. reserv.
; 0 (Viral Proteins); 9007-49-2 (DNA)
Enzyme No.: EC 2.7.7.- (Cre recombinase); EC 2.7.7.- (Integrase)
Record Date Created: 20010820
Record Date Completed: 20010913

- end of record -

?
Display 6/9/7 (Item 1 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)

(c) 2003 American Chemical Society. All rts. reserv.

139112191 CA: 139(8)112191h JOURNAL
Integration by design
AUTHOR(S): Sandmeyer, Suzanne
LOCATION: Department of Biological Chemistry, College of Medicine, University of California, Irvine, CA, 92697-1700, USA
JOURNAL: Proc. Natl. Acad. Sci. U. S. A. (Proceedings of the National Academy of Sciences of the United States of America) DATE: 2003 VOLUME: 100 NUMBER: 10 PAGES: 5586-5588 CODEN: PNASA6 ISSN: 0027-8424
LANGUAGE: English PUBLISHER: National Academy of Sciences
SECTION:
CA203000 Biochemical Genetics
CA207XXX Enzymes
CA210XXX MICROBIAL, ALGAL, AND FUNGAL BIOCHEMISTRY
IDENTIFIERS: review commentary retroviral integration modified integrase, retrotransposon Ty5 modified integrase control integration review commentary

-more-

?
Display 6/9/7 (Item 1 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)
(c) 2003 American Chemical Society. All rts. reserv.
DESCRIPTORS:
Recombination,genetic...
integration; understanding mechanisms and implication of retroviral integration, and strategy for retargeting Ty5 integration
Retrotransposon...
Ty5; understanding mechanisms and implications of retroviral integration, and approach for controlling Saccharomyces Ty5 integration using modified integrase
Retroviral vectors...
understanding mechanisms and implication of retroviral integration, and implications of findings on retroviral vector gene therapy
Retroviridae...
understanding mechanisms and implication of retroviral integration, and strategy for retargeting Ty5 integration
Saccharomyces...
understanding mechanisms and implications of retroviral integration, and approach for controlling Saccharomyces Ty5 integration using

-more-

?
Display 6/9/7 (Item 1 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)
(c) 2003 American Chemical Society. All rts. reserv.
modified integrase
CAS REGISTRY NUMBERS:
52350-85-3 understanding mechanisms and implications of retroviral integration, and approach for controlling Ty5 integration using modified integrase

- end of record -

?
Display 6/9/8 (Item 1 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
(c) 2003 Inst for Sci Info. All rts. reserv.

09909230 Genuine Article#: 464BL Number of References: 38
Title: Examining human T-lymphotropic virus type 1 infection and replication by cell-free infection with recombinant virus vectors
Author(s): Derse D (REPRINT) ; Hill SA; Lloyd PA; Chung HK; Morse BA

Corporate Source: NCI,Basic Res Lab,Bldg 567/Frederick//MD/21702 (REPRINT); NCI,Basic Res Lab,Frederick//MD/21702; SAIC Frederick,Frederick//MD/21702
Journal: JOURNAL OF VIROLOGY, 2001, V75, N18 (SEP), P8461-8468
ISSN: 0022-538X Publication date: 20010900
Publisher: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC 20036-2904 USA
Language: English Document Type: ARTICLE
Geographic Location: USA
Journal Subject Category: VIROLOGY
Abstract: A sensitive and quantitative cell-free infection assay, utilizing recombinant human T-cell leukemia virus type 1 (HTLV-1)-based vectors,

-more-

?
Display 6/9/8 (Item 1 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

was developed in order to analyze early events in the virus replication cycle. Previous difficulties with the low infectivity and restricted expression of the virus have prevented a clear understanding of these events. Virus stocks were generated by transfecting cells with three plasmids: (i) a packaging plasmid encoding HTLV-1 structural and regulatory proteins, (ii) an HTLV-1 transfer vector containing either firefly luciferase or enhanced yellow fluorescent protein genes, and (iii) an envelope expression plasmid. Single-round infections were initiated by exposing target cells to filtered supernatants and quantified by assaying for luciferase activity in cell extracts or by enumerating transduced cells by flow cytometry. Transduction was dependent on reverse transcription and integration of the recombinant virus genome, as shown by the effects of the reverse transcriptase inhibitor 3 ' -azido-3 ' -deoxythymidine (AZT) and by **mutation** of the **integrase** gene in the packaging vector, respectively. The 50% inhibitory concentration of AZT was determined to be 30 nM in this HTLV-1 replication system. The stability of HTLV-1 particles,

-more-

?
Display 6/9/8 (Item 1 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

pseudotyped with either vesicular stomatitis virus G protein or HTLV-1 envelope, was typical of retroviruses, exhibiting a half-life of approximately 3.5 h at 37 degreesC. The specific infectivity of recombinant HTLV-1 virions was at least 3 orders of magnitude lower than that of analogous HIV-1 particles, though both were pseudotyped with the same envelope. Thus, the low infectivity of HTLV-1 is determined in large part by properties of the core particle and by the efficiency of postentry processes.
Identifiers--KeyWord Plus(R): LYMPHADENOPATHY-ASSOCIATED VIRUS; TROPICAL SPASTIC PARAPARESIS; BLOOD MONONUCLEAR-CELLS; HUMAN-ENDOTHELIAL CELLS; LEUKEMIA-VIRUS; PERIPHERAL-BLOOD; HTLV-I; SURFACE PHENOTYPE; **LENTIVIRAL VECTOR**; MOLECULAR CLONE
Cited References:
BOUCHER CAB, 1992, V165, P105, J INFECT DIS
CLAPHAM P, 1983, V222, P1125, SCIENCE
DELAMARRE L, 1997, V71, P259, J VIROL
DEROSSI A, 1985, V143, P640, VIROLOGY

-more-

?
Display 6/9/8 (Item 1 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

DERSE D, 1996, V12, P1, J ACQ IMMUN DEF SYND
DERSE D, 1995, V69, P1907, J VIROL
DULL T, 1998, V72, P8463, J VIROL
FAN N, 1992, V30, P905, J CLIN MICROBIOL
FRANCHINI G, 1995, V86, P3619, BLOOD
GESSAIN A, 1985, V2, P407, LANCET
GESSAIN A, 1990, V71, P333, J GEN VIROL
HATTORI T, 1981, V58, P645, BLOOD
HIGASHIKAWA F, 2001, V280, P124, VIROLOGY
HO DD, 1984, V81, P7588, P NATL ACAD SCI USA
HOXIE JA, 1984, V81, P7591, P NATL ACAD SCI USA
ISONO T, 1990, V14, P841, LEUKEMIA RES
LANDAU NR, 1991, V65, P162, J VIROL
LONGO DL, 1984, V310, P505, NATURE
MACCHI B, 1997, V78, P1007, J GEN VIROL 5
MANN DL, 1984, V74, P56, J CLIN INVEST
MANSKY LM, 2000, V74, P9525, J VIROL

-more-

?

Display 6/9/8 (Item 1 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

MATSUSHITA S, 1987, V80, P394, J CLIN INVEST
MITSUYA H, 1985, V82, P7096, P NATL ACAD SCI USA
NAKASHIMA H, 1986, V30, P933, ANTIMICROB AGENTS CH
NALDINI L, 1996, V93, P11382, P NATL ACAD SCI USA
NALDINI L, 1996, V272, P263, SCIENCE
OSAME M, 1986, V1, P1031, LANCET
POIESZ BJ, 1980, V77, P7415, P NATL ACAD SCI USA
POWELL SK, 1999, V73, P8813, J VIROL
RICHARDSON JH, 1990, V64, P5682, J VIROL
SHUH M, 1999, V262, P442, VIROLOGY
SOMMERFELT MA, 1988, V242, P1557, SCIENCE
STAIR RK, 1991, V65, P6339, J VIROL
SUTTON RE, 1996, V70, P7322, J VIROL
TREJO SR, 2000, V268, P41, VIROLOGY
VILE RG, 1991, V180, P420, VIROLOGY
WILSON C, 1989, V63, P2374, J VIROL
YOSHIDA M, 1982, V12, P285, PRINCESS TAKAMATSU S

- end of record -

?

Display 6/9/9 (Item 2 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

09098892 Genuine Article#: 367EH Number of References: 42
Title: Critical factors influencing stable transduction of human CD34(+) cells with HIV-1-derived **lentiviral vectors**
Author(s): Haas DL; Case SS; Crooks GM; Kohn DB (REPRINT)
Corporate Source: CHILDRENS HOSP LOS ANGELES,DIV RES IMMUNOL BONE MARROW TRANSPLANTAT, 4650 SUNSET BLVD MAILSTOP 62/LOS ANGELES//CA/90027 (REPRINT); CHILDRENS HOSP LOS ANGELES,DIV RES IMMUNOL BONE MARROW TRANSPLANTAT/LOS ANGELES//CA/90027; UNIV SO CALIF,SCH MED, DEPT MOL MICROBIOL & IMMUNOL/LOS ANGELES//CA/90033
Journal: MOLECULAR THERAPY, 2000, V2, N1 (JUL), P71-80
ISSN: 1525-0016 Publication date: 20000700
Publisher: ACADEMIC PRESS INC, 525 B ST, STE 1900, SAN DIEGO, CA 92101-4495
Language: English Document Type: ARTICLE
Geographic Location: USA
Subfile: CC LIFE--Current Contents, Life Sciences

Journal Subject Category: BIOTECHNOLOGY & APPLIED MICROBIOLOGY; GENETICS &

-more-

?
Display 6/9/9 (Item 2 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

HEREDITY; MEDICINE, RESEARCH & EXPERIMENTAL
Abstract: **Lentiviral vectors** have been proposed as a more efficient alternative to Moloney murine leukemia virus-based **retroviral vectors** for transduction of human hematopoietic progenitors and stem cells. These studies were designed to evaluate the conditions that influence transduction frequency of CD34(+) progenitors, with the goal of optimizing efficiency of stable gene transfer with **lentiviral vectors**. CD34(+) human cord blood cells and 293 cells were transduced with a human immunodeficiency virus (HIV)-1 derived **lentiviral vector** pseudotyped with vesicular stomatitis virus glycoprotein and carrying an internal human cytomegalovirus promoter driving enhanced green fluorescent protein (eGFP) expression. Using fluorescence-activated cell sorting analysis of eGFP, we observed pseudotransduction beginning at the time of vector addition and lasting up to 24 h in CD34(+) cells and up to 72 h in 293 cells. **Integrase-defective lentiviral vector** caused transient eGFP expression for up to 10 days in CD34(+) cells and

-more-

?
Display 6/9/9 (Item 2 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
(c) 2003 Inst for Sci Info. All rts. reserv.
for up to 14 days in 293 cells. Protamine sulfate conferred no increase in transduction efficiency of CD34(+) cells on fibronectin-coated plates. Transduction frequency was related directly to vector concentration and not to multiplicity of infection across the ranges tested. First- and second-generation **lentiviral vectors** transduced CD34(+) cells equally, demonstrating a lack of dependence on HIV-1 accessory proteins. These findings will be useful for the optimal utilization of this new class of vectors for transduction of human hematopoietic stem cells.
Descriptors--Author Keywords: **lentiviral vector** ; CD34 ; transduction ; hematopoietic stem cells ; gene therapy
Identifiers--KeyWord Plus(R): HEMATOPOIETIC STEM-CELLS; EX-VIVO EXPANSION; EFFICIENCY GENE-TRANSFER; MURINE LEUKEMIA-VIRUS; **RETROVIRAL VECTORS**; PROGENITOR CELLS; BONE-MARROW; IN-VIVO; REPOPULATING ABILITY; GROWTH-FACTORS
Cited References:
AKKINA RK, 1996, V70, P2581, J VIROL

-more-

?
Display 6/9/9 (Item 2 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
(c) 2003 Inst for Sci Info. All rts. reserv.
BLOMER U, 1997, V71, P6641, J VIROL
BODINE DM, 1991, V78, P914, BLOOD
BURNS JC, 1993, V90, P8033, P NATL ACAD SCI USA
CASE SS, 1999, V96, P2988, P NATL ACAD SCI USA
CHALFIE M, 1994, V263, P802, SCIENCE
CHUCK AS, 1996, V7, P1527, HUM GENE THER
CONTI C, 1991, V142, P17, RES VIROLOGY
CROOKS GM, 1993, V82, P3290, BLOOD
DAO MA, 1998, V92, P4612, BLOOD
DULL T, 1998, V72, P8463, J VIROL

GAFFAY P, 1995, V83, P569, CELL
GALLARDO HF, 1997, V90, P952, BLOOD
GOTHOT A, 1998, V92, P2641, BLOOD
HANENBERG H, 1997, V8, P2193, HUM GENE THER
HAO QL, 1995, V86, P3745, BLOOD
HAO QL, 1996, V88, P3306, BLOOD
HARRISON DE, 1987, V69, P1021, BLOOD

-more-

?
Display 6/9/9 (Item 2 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

JONES RJ, 1990, V347, P188, NATURE
LAROCHELLE A, 1996, V2, P1329, NAT MED
LI X, 1998, V273, P34970, J BIOL CHEM
LIU ML, 1996, V70, P2497, J VIROL
MASTROMARINO P, 1987, V68, P2359, J GEN VIROL
MIYOSHI H, 1999, V283, P682, SCIENCE
MORGAN JR, 1995, V69, P6994, J VIROL
MORITZ T, 1996, V88, P855, BLOOD
MORITZ T, 1994, V93, P1451, J CLIN INVEST
NALDINI L, 1996, V93, P11382, P NATL ACAD SCI USA
NALDINI L, 1996, V272, P263, SCIENCE
ORLIC D, 1996, V93, P11097, P NATL ACAD SCI USA
PETERS SO, 1996, V87, P30, BLOOD
PLOEMACHER RE, 1989, V74, P2755, BLOOD
PLOEMACHER RE, 1991, V78, P2527, BLOOD
ROBBINS PB, 1998, V95, P10182, P NATL ACAD SCI USA
SONEOKA Y, 1995, V23, P628, NUCLEIC ACIDS RES

-more-

?
Display 6/9/9 (Item 2 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

SUTTON RE, 1998, V72, P5781, J VIROL
SUTTON RE, 1999, V73, P3649, J VIROL
TISDALE JF, 1998, V92, P1131, BLOOD
TRAYCOFF CM, 1995, V85, P2059, BLOOD
UCHIDA N, 1998, V95, P11939, P NATL ACAD SCI USA
YOKOTA T, 1998, V91, P3263, BLOOD
ZUFFEREY R, 1997, V15, P871, NAT BIOTECHNOL

- end of record -

?
Display 6/9/10 (Item 3 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

07140547 Genuine Article#: 127WN Number of References: 65
Title: High-titer human immunodeficiency virus type 1-based vector systems for gene delivery into nondividing cells
Author(s): Mochizuki H; Schwartz JP; Tanaka K; Brady RO; Reiser J (REPRINT)
Corporate Source: NINCDS,MOL & MED GENET SECT, DEV & METAB NEUROL BRANCH, NIH, BLDG 10, ROOM 3D04, 1/BETHESDA//MD/20892 (REPRINT); NINCDS,MOL & MED GENET SECT, DEV & METAB NEUROL BRANCH, NIH/BETHESDA//MD/20892; NINCDS,MOL GENET SECT, CLIN NEUROSCI BRANCH, NIH/BETHESDA//MD/20892; NHLBI,CARDIOL BRANCH, NIH/BETHESDA//MD/20892
Journal: JOURNAL OF VIROLOGY, 1998, V72, N11 (NOV), P8873-8883
ISSN: 0022-538X Publication date: 19981100
Publisher: AMER SOC MICROBIOLOGY, 1325 MASSACHUSETTS AVENUE, NW,

WASHINGTON, DC 20005-4171
Language: English Document Type: ARTICLE
Geographic Location: USA

-more-

?
Display 6/9/10 (Item 3 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
(c) 2003 Inst for Sci Info. All rts. reserv.
Subfile: CC LIFE--Current Contents, Life Sciences
Journal Subject Category: VIROLOGY
Abstract: Previously we designed novel pseudotyped high-titer replication defective human immunodeficiency virus type 1 (HIV-1) vectors to deliver genes into nondividing cells (J. Reiser, G. Harmison, S. Kluepfel-Stahl, R. O. Brady, S. Karlsson, and M. Schubert, Proc. Natl. Acad. Sci. USA 93:15266-15271, 1996). Since then we have made several improvements with respect to the safety, flexibility, and efficiency of the vector system. A three-plasmid expression system is used to generate pseudotyped HIV-1 particles by transient transfection of human embryonic kidney 293T cells with a defective packaging construct, a plasmid coding for a heterologous envelope (Env) protein, and a vector construct harboring a reporter gene such as neo, ShlacZ (encoding a phleomycin resistance/beta-galactosidase fusion protein), HSA (encoding mouse heat-stable antigen), or EGFP (encoding enhanced green fluorescent protein). The packaging constructs lack functional Vif, Vpr, and Vpu proteins and/or a large portion of the Env coding region

-more-

?
Display 6/9/10 (Item 3 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
(c) 2003 Inst for Sci Info. All rts. reserv.
as well as the 5' and 3' long terminal repeats, the Nef function, and the presumed packaging signal. Using G418 selection, we routinely obtained vector particles pseudotyped with the vesicular stomatitis virus G glycoprotein (VSV-G) with titers of up to 8 x 10(7) CFU/mu g of p24, provided that a functional Tat coding region was present in the vector. Vector constructs lacking a functional Tat protein yielded titers of around 4 x 10(6) to 8 x 10(6) CFU/mu g of p24. Packaging constructs with a **mutation** within the **integrase** (IN) core domain profoundly affected colony formation and expression of the reporter genes, indicating that a functional IN protein is required for efficient transduction. We explored the abilities of other Env proteins to allow formation of pseudotyped HIV-1 particles, The rabies virus and Mokola virus G proteins yielded high-titer infectious pseudotypes, while the human foamy virus Env protein did not. Using the improved vector system, we successfully transduced contact-inhibited primary human skin fibroblasts and postmitotic rat cerebellar neurons and cardiac myocytes, a process not affected by the lack of the accessory

-more-

?
Display 6/9/10 (Item 3 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
(c) 2003 Inst for Sci Info. All rts. reserv.
proteins.
Identifiers--KeyWord Plus(R): **RETROVIRAL VECTOR**; HUMAN-LYMPHOCYTES; MURINE **RETROVIRUSES**; NUCLEOTIDE-SEQUENCE; **LENTIVIRAL VECTOR**; AIDS VIRUS; IN-VIVO; HIV; EXPRESSION; GLYCOPROTEIN
Cited References:
ADACHI A, 1986, V59, P284, J VIROL
AKKINA RK, 1996, V70, P2581, J VIROL

BARON M, 1992, V114, P239, GENE
BOSHART M, 1985, V41, P521, CELL
BUCHSCHACHER GL, 1992, V66, P2731, J VIROL
CANIVET M, 1990, V178, P543, VIROLOGY
CARROLL R, 1994, V68, P6047, J VIROL
CHANG HK, 1995, V2, P189, J BIOMED SCI
CHESEBRO B, 1990, V64, P4553, J VIROL
CLEVER JL, 1997, V71, P3407, J VIROL
CORBEAU P, 1996, V93, P14070, P NATL ACAD SCI USA

-more-

?
Display 6/9/10 (Item 3 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

DUBRIDGE RB, 1987, V7, P379, MOL CELL BIOL
DUNBAR CE, 1996, V47, P11, ANNU REV MED
EMERMAN M, 1996, V14, P943, NAT BIOTECHNOL
ENGLUND G, 1995, V69, P3216, J VIROL
FLUGEL RM, 1987, V6, P2077, EMBO J
GIBBS JS, 1994, V10, P343, AIDS RES HUM RETROV
GOEPFERT PA, 1997, V71, P778, J VIROL
HUANG LM, 1994, V13, P2886, EMBO J
KAFRI T, 1997, V17, P314, NAT GENET
KIM VN, 1998, V72, P811, J VIROL
KIMPTON J, 1992, V66, P2232, J VIROL
LANDAU NR, 1991, V65, P162, J VIROL
LEVER A, 1989, V63, P4085, J VIROL
LEVER AML, 1996, V3, P470, GENE THER
LEWIS P, 1992, V11, P3053, EMBO J
LEWIS PF, 1994, V68, P510, J VIROL
LINDEMANN D, 1997, V71, P4815, J VIROL

-more-

?
Display 6/9/10 (Item 3 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

LIU ML, 1996, V70, P2497, J VIROL
LUSSO P, 1990, V247, P848, SCIENCE
MANN DL, 1989, V5, P253, AIDS RES HUM RETROV
MCBRIDE MS, 1996, V70, P2963, J VIROL
MEBATSION T, 1995, V69, P1444, J VIROL
MILLER AD, 1990, V1, P5, HUM GENE THER
MILLER DG, 1990, V10, P4239, MOL CELL BIOL
MOCHIZUKI H, UNPUB
MYERS G, 1996, HUMAN RETROVIRUSES A
NALDINI L, 1996, V272, P263, SCIENCE
OTT D, 1990, V64, P757, J VIROL
PAGE KA, 1990, V64, P5270, J VIROL
PAROLIN C, 1994, V68, P3888, J VIROL
PAROLIN C, 1996, V222, P415, VIROLOGY
PEAR WS, 1993, V90, P8392, P NATL ACAD SCI USA
POESCHLA E, 1996, V93, P11395, P NATL ACAD SCI USA
POZNANSKY M, 1991, V65, P532, J VIROL

-more-

?
Display 6/9/10 (Item 3 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

PRACYK JB, 1997, V122, P404, SURGERY
RATNER L, 1987, V3, P57, AIDS RES HUM RETROV

RATNER L, 1985, V313, P277, NATURE
REISER J, 1996, V93, P15266, P NATL ACAD SCI USA
RICHARDSON JH, 1995, V76, P691, J GEN VIROL
ROE TY, 1993, V12, P2099, EMBO J
SCHNEIDER R, 1997, V71, P4892, J VIROL
SHIMADA T, 1991, V88, P1043, J CLIN INVEST
SONEOKA Y, 1995, V23, P628, NUCLEIC ACIDS RES
SPECTOR DH, 1990, V64, P2298, J VIROL
SPRINGETT GM, 1989, V63, P3865, J VIROL
SRINIVASAKUMAR N, 1997, V71, P5841, J VIROL
STEVENSON M, 1996, V6, P9, TRENDS CELL BIOL
STRAIR RK, 1993, V21, P4836, NUCLEIC ACIDS RES
TANIWAKI T, 1995, V64, P2509, J NEUROCHEM
TOBEY RA, 1988, V179, P400, EXP CELL RES
VERMA IM, 1997, V389, P239, NATURE

-more-

?
Display 6/9/10 (Item 3 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

YU H, 1996, V70, P4530, J VIROL
ZHU Z, 1990, V3, P215, J ACQ IMMUN DEF SYND
ZUFFEREY R, 1997, V15, P871, NAT BIOTECHNOL

- end of record -

?
Display 6/9/11 (Item 4 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

04910144 Genuine Article#: UR130 Number of References: 52
Title: FUNCTIONAL DOMAINS OF MOLONEY MURINE LEUKEMIA-VIRUS **INTEGRASE** DEFINED BY **MUTATION** AND COMPLEMENTATION ANALYSIS
Author(s): JONSSON CB; DONZELLA GA; GAUCAN E; SMITH CM; ROTH MJ
Corporate Source: UNIV MED & DENT NEW JERSEY,ROBERT WOOD JOHNSON MED SCH,DEPT BIOCHEM,675 HOES LN/PISCATAWAY//NJ/08854; UNIV MED & DENT NEW JERSEY,ROBERT WOOD JOHNSON MED SCH,DEPT BIOCHEM/PISCATAWAY//NJ/08854
Journal: JOURNAL OF VIROLOGY, 1996, V70, N7 (JUL), P4585-4597
ISSN: 0022-538X
Language: ENGLISH Document Type: ARTICLE
Geographic Location: USA
Subfile: SciSearch; CC LIFE--Current Contents, Life Sciences
Journal Subject Category: VIROLOGY
Abstract: Retroviral integrases perform two catalytic steps, 3' processing and strand transfer, that result in the stable insertion of the retroviral DNA into the host genome. Mutant M-MuLV integrases were

-more-

?
Display 6/9/11 (Item 4 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

constructed to define the functional domains important for 3' processing, strand transfer, and disintegration by in vitro assays. N-terminal mutants had no detectable 3' processing activity, and only one mutant which lacks the HHCC domain, N Delta 105, had strand transfer activity. Strand transfer mediated by N Delta 105 showed preference for one site in the target DNA. Disintegration activity of N-terminal mutants decreased only minimally. In contrast, all C-terminal mutants truncated by more than 28 amino acids had no integration or disintegration activity. Activity on a single-strand disintegration substrate did not require a functional HHCC domain but

did require most of the C-terminal region. Complementation analysis found that the HHCC region alone was able to function in trans to a promoter containing only the DD(35)E and C-terminal regions and to enhance integration site selection. Increasing the reducing conditions or adding the HHCC domain to N Delta 105 reaction mixtures restored the wild-type strand transfer activity and range of target sites. The reducing agent affected Cys-209 in the DD(35)E region. The presence of

-more-

?
Display 6/9/11 (Item 4 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

C-209 was required for complementation of N Delta 105 by the HHCC region.
Identifiers--KeyWords Plus: HUMAN-IMMUNODEFICIENCY-VIRUS; SITE-DIRECTED MUTAGENESIS; DNA-BINDING; HIV-1 INTEGRASE; VIRAL-DNA; RETROVIRUS INTEGRASE; PROTEIN; TYPE-1; IDENTIFICATION; SEQUENCES
Research Fronts: 94-0947 001 (**RETROVIRAL VECTOR**-MEDIATED GENE-TRANSFER; HUMAN HEMATOPOIETIC PROGENITOR CELLS; EFFICIENT TRANSDUCTION; PERIPHERAL-BLOOD LYMPHOCYTES)
94-3070 001 (RAT SKELETAL-MUSCLE; DEVELOPMENTAL REGULATION; YEAST SACCHAROMYCES-CEREVISIAE)
Cited References:
BOWERMAN B, 1989, V3, P469, GENE DEV
BRADFORD MM, 1976, V72, P248, ANAL BIOCHEM
BUSHMAN FD, 1994, V68, P2215, J VIROL
BUSHMAN FD, 1993, V90, P3428, P NATL ACAD SCI USA
CHOW SA, 1995, V68, P7869, J VIROL
CHOW SA, 1992, V255, P723, SCIENCE

-more-

?
Display 6/9/11 (Item 4 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

COFFIN JM, 1990, P645, FUNDAMENTAL VIROLOGY
COLICELLI J, 1985, V42, P573, CELL
CRAIGIE R, 1990, V62, P829, CELL
DONEHOWER LA, 1988, V62, P3958, J VIROL
DONZELLA GA, 1993, V67, P7077, J VIROL
DOTAN I, 1995, V69, P456, J VIROL
ELLISON V, 1995, V270, P3320, J BIOL CHEM
ENGELMAN A, 1991, V67, P1211, CELL
ENGELMAN A, 1993, V12, P3269, EMBO J
ENGELMAN A, 1992, V66, P6361, J VIROL
FARNET CM, 1991, V65, P1910, J VIROL
FELKNER RH, 1992, V66, P4258, J VIROL
HO SN, 1989, V77, P51, GENE
JOHNSON MS, 1986, V83, P7648, P NATL ACAD SCI USA
JONSSON CB, 1993, V268, P1462, J BIOL CHEM
JONSSON CB, 1993, V67, P5562, J VIROL
KATZ RA, 1994, V63, P133, ANN REV BIOCH

-more-

?
Display 6/9/11 (Item 4 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

KATZMAN M, 1989, V63, P5319, J VIROL
KHAN E, 1990, V19, P851, NUCLEIC ACIDS RES
KULKOSKY J, 1992, V12, P2331, MOL CELL BIOL
KUNKEL TA, 1987, V154, P367, METHOD ENZYMOL

LEAVITT AD, 1993, V268, P2113, J BIOL CHEM
LIN TH, 1989, V5, P156, PROTEINS
MAZUMDER A, 1994, V22, P1037, NUCLEIC ACIDS RES
MILLER DG, 1990, V10, P4239, MOL CELL BIOL
MUMM SR, 1991, V65, P1160, J VIROL
MURPHY JE, 1992, V66, P5092, J VIROL
MURPHY JE, 1993, V195, P432, VIROLOGY
PURASLUTZKE R, 1994, V22, P4125, NUCLEIC ACIDS RES
ROE TY, 1993, V12, P2099, EMBO J
ROTH MJ, 1989, V58, P47, CELL
ROTH MJ, 1990, V64, P4709, J VIROL
ROTH MJ, 1991, V65, P2141, J VIROL
SANGER F, 1978, V74, P5463, P NATL ACAD SCI USA

-more-

?
Display 6/9/11 (Item 4 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

SCHWARTZBERG PL, 1993, V192, P673, VIROLOGY
SHINNICK TM, 1981, V293, P543, NATURE
VANDENENT FL, 1995, V68, P7825, J VIROL
VANGENT DC, 1993, V12, P3261, EMBO J
VANGENT DC, 1991, V19, P3821, NUCLEIC ACIDS RES
VANGENT DC, 1992, V89, P9598, P NATL ACAD SCI USA
VARMUS H, 1989, RETROVIRUSES
VINK C, 1993, V21, P1419, NUCLEIC ACIDS RES
VINK C, 1993, V19, P6691, NUCLEIC ACIDS RES
VOGELSTEIN B, 1979, V76, P615, P NATL ACAD SCI USA
WOERNER AM, 1992, V8, P297, AIDS RES HUM RETROV
WOERNER AM, 1993, V21, P3507, NUCLEIC ACIDS RES

- end of record -

?
Display 6/9/12 (Item 1 from file: 71)
DIALOG(R)File 71:ELSEVIER BIOBASE

01111416 1999075226
Stable transduction of quiescent CD34sup +CD38sup - human hematopoietic cells by HIV-1-based **lentiviral vectors**
Case S.S.; Price M.A.; Jordan C.T.; Xiao Jin Yu; Wang L.; Bauer G.; Haas D.L.; Xu D.; Stripecke R.; Naldini L.; Kohn D.B.; Crooks G.M.
ADDRESS: G.M. Crooks, Childrens Hospital Los Angeles, MS 62, 4650 Sunset Blvd., Los Angeles, CA 90027, United States
EMAIL: gcrooks@chla.usc.edu
Journal: Proceedings of the National Academy of Sciences of the United States of America, 96/6 (2988-2993), 1999, United States
CODEN: PNASA
ISSN: 0027-8424
DOCUMENT TYPE: Conference Paper
LANGUAGES: English SUMMARY LANGUAGES: English
NO. OF REFERENCES: 52

-more-

?
Display 6/9/12 (Item 1 from file: 71)
DIALOG(R)File 71:ELSEVIER BIOBASE

We compared the efficiency of transduction by an HIV-1-based **lentiviral vector** to that by a Moloney murine leukemia virus (MLV) **retroviral vector**, using stringent in vitro assays of primitive, quiescent human hematopoietic progenitor cells. Each construct

contained the enhanced green fluorescent protein (GFP) as a reporter gene. The **lentiviral vector**, but not the MLV vector, expressed GFP in nondivided CD34sup + cells (45.5% GFPsup +) and in CD34sup +CD38sup - cells in Ginf 0 (12.4% GFPsup +), 48 hr after transduction. However, GFP could also be detected short-term in CD34sup + cells transduced with a **lentiviral vector** that contained a **mutated integrase** gene. The level of stable transduction from integrated vector was determined after extended long-term bone marrow culture. Both MLV **vectors** and **lentiviral vectors** efficiently transduced cytokine-stimulated CD34sup + cells. The MLV vector did not transduce more primitive, quiescent CD34sup +CD38sup - cells (n = 8). In contrast, stable transduction of CD34sup +CD38sup - cells by the **lentiviral vector** was seen for over 15 weeks of extended long-term culture (9.2

-more-

?
Display 6/9/12 (Item 1 from file: 71)
DIALOG(R)File 71:ELSEVIER BIOBASE
(c) 2003 Elsevier Science B.V. All rts. reserv.
+/- 5.2%, n = 7). GFP expression in clones from single CD34sup +CD38sup - cells confirmed efficient, stable lentiviral transduction in 29% of early and late- proliferating cells. In the absence of growth factors during transduction, only the **lentiviral vector** was able to transduce CD34sup + and CD34sup +CD38sup - cells (13.5 +/- 2.5%, n = 11 and 12.2 +/- 9.7%, n = 4, respectively). The **lentiviral vector** is clearly superior to the MLV vector for transduction of quiescent, primitive human hematopoietic progenitor cells and may provide therapeutically useful levels of gene transfer into human hematopoietic stem cells.

CLASSIFICATION CODE AND DESCRIPTION:
86.5.4.6 - IMMUNOLOGY AND INFECTIOUS DISEASES / HUMORAL MEDIATORS OF IMMUNE RESPONSE / Other Factors / Haematopoietic growth factors (CSF)
86.7.7.1 - IMMUNOLOGY AND INFECTIOUS DISEASES / IMMUNITY TO INFECTION / AIDS and HIV / Diagnosis and disease monitoring
86.7.7.4 - IMMUNOLOGY AND INFECTIOUS DISEASES / IMMUNITY TO INFECTION / AIDS and HIV / Pathogenesis and syndrome manifestations

-more-

?
Display 6/9/12 (Item 1 from file: 71)
DIALOG(R)File 71:ELSEVIER BIOBASE
(c) 2003 Elsevier Science B.V. All rts. reserv.
86.7.7.5 - IMMUNOLOGY AND INFECTIOUS DISEASES / IMMUNITY TO INFECTION / AIDS and HIV / Infectious complications

- end of record -

?
Display 6/9/13 (Item 1 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.
(c) 2003 Thomson Derwent & ISI. All rts. reserv.

0314050 DBR Accession No.: 2003-15190 PATENT
New non-mouse packaging cell line useful as drug transport systems, particularly for delivering vector constructs or therapeutics to cells, produces a ten-fold increase or more in viral packaging protein compared to normal - packaging cell culture and vector expression in host cell for use in gene therapy
AUTHOR: BARBER J R; JOLLY D J; RESPESS J G; CHANG S M W
PATENT ASSIGNEE: BARBER J R; JOLLY D J; RESPESS J G; CHANG S M W 2003
PATENT NUMBER: US 20030003567 PATENT DATE: 20030102 WPI ACCESSION NO.: 2003-361917 (200334)
PRIORITY APPLIC. NO.: US 205179 APPLIC. DATE: 20020724
NATIONAL APPLIC. NO.: US 205179 APPLIC. DATE: 20020724

LANGUAGE: English
ABSTRACT: DERWENT ABSTRACT: NOVELTY - A non-mouse packaging cell line, which produces at least a ten-fold increase in viral packaging protein as compared to a standard mouse amphotropic packaging cell line, is

-more-

?
Display 6/9/13 (Item 1 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.
(c) 2003 Thomson Derwent & ISI. All rts. reserv.
new. DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) Selecting packaging cells, which produce high levels of a primary agent selected from a packaging protein or a gene product; (2) Producing a recombinant retrovirus; (3) A hybrid env gene useful for preparing a retrovirus that can selectively carry a gene to a target cell, where the env gene codes for: (a) a cytoplasmic segment of a first retroviral phenotype; and (b) a binding segment exogenous to the first retroviral phenotype, the binding segment capable of selectively binding to the target cell; (4) Producing a transgenic packaging animal or insect; (5) A cell line consisting of CA, 2A, DA, DA2, DX, HX or HP (not defined); and (6) Producing a vector capable of infecting a cell type. BIOTECHNOLOGY - Preferred Packaging Cell Line: The packaging cell line is amphotropic, polytropic, or xenotropic. The viral packaging protein is the gag/pol protein. The non-mouse packaging cell line carries on separate operons the genes for gag/pol and env. The operons lack retroviral LTR sequences and, upon introduction of an N2 type vector construct, produces substantially no helper virus after

-more-

?
Display 6/9/13 (Item 1 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.
(c) 2003 Thomson Derwent & ISI. All rts. reserv.
at least twenty days passage in culture. The cell line is an amphotropic packaging cell line, a polytropic packaging cell line, or a xenotropic packaging cell line. The packaging cell line, upon introduction of vector construct, produces at least a ten-fold increase in vector titer as compared to a standard mouse amphotropic packaging cell line. The packaging cell line, upon introduction of a vector construct, produces vector particles capable of infecting human cells. The xenotropic packaging cell line, upon introduction of a vector construct, is capable of producing vector particles substantially uncontaminated by replication competent virus. In particular, the cell line produces at least equal vector titer as compared to a standard mouse amphotropic packaging cell line when HT1080 cells are infected. The polytropic packaging cell line, upon introduction of a vector construct, produces at least a ten-fold increase in vector titer as compared to a standard mouse amphotropic packaging cell line when 293 cells are infected. The packaging cell line, upon introduction of a vector construct, produces vector particles capable of infecting cells

-more-

?
Display 6/9/13 (Item 1 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.
(c) 2003 Thomson Derwent & ISI. All rts. reserv.
of kidney lineage, but not cells of fibroblast, epithelial, T cell or monocyte lineage. Preferred Method: In method (1), selecting packaging cells, which produce high levels of a primary agent selected from a packaging protein or a gene product, comprises: (a) providing in packaging cells a genome comprising a primary gene that expresses a primary agent in it, and a selectable gene that expresses a selectable protein in it at lower levels than the primary agent, the expression

levels of the primary gene and selectable being proportional; (b) exposing the packaging cells to a selecting agent that enables identification of those cells that express the selectable protein at a critical level; and (c) detecting those packaging cells that express high levels of the primary agent. In method (2), producing a recombinant retrovirus comprises: (a) generating a gag, pol or env proteins from a cell line infected by a recombinant virus, which is capable of producing the proteins; and (b) contacting the proteins with viral vector RNA, tRNA, liposomes, and a cell extract to complement missing functions for particle assembly, so as to produce recombinant

-more-

?

Display 6/9/13 (Item 1 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.
(c) 2003 Thomson Derwent & ISI. All rts. reserv.

retroviruses carrying the viral **vector** RNA. The method also comprises: (a) generating recombinant viral vectors, which separately or in combination, code for gag/pol, env and a **retroviral vector** genome; (b) producing high titer stocks of the vectors; and (c) co-infecting primary or other cells to generate recombinant **retroviral vectors**. Producing a recombinant **retrovirus** also involves growing a producer cell line having a genome with: (a) a gene of interest along with a packaging signal of a first retroviral phenotype; (b) gag and pol genes of the first retroviral phenotype, absent a packaging signal; and a hybrid env gene absent a packaging signal, the product of the hybrid env gene comprising a cytoplasmic segment of the first retroviral phenotype, and a binding segment exogenous to the first retroviral phenotype, or a gene coding for a ligand (particularly CD4), which is expressed on the surface of the producer cell, and which is subsequently exhibited on the surface of the vector particle.Producing a recombinant retrovirus, which is capable of integrating its genome into a preselected site on a

-more-

?

Display 6/9/13 (Item 1 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.
(c) 2003 Thomson Derwent & ISI. All rts. reserv.

target cell's genome, comprises packaging a vector in a capsid and envelope; and including in a viral particle a **modified** form of **integrase** that is capable of integrating the retroviral genome into the preselected site. Producing a recombinant retrovirus also comprises: (a) mating a transgenic animal or insect containing a gag/pol-env viral construct, with a transgenic animal or insect containing a vector construct with a promoter; (b) isolating the progeny of the transgenic animals or insects; (c) isolating selected cells from the progeny; (d) growing the cells in an appropriate medium; and (e) isolating recombinant retroviruses from the cells. The method also comprises: (a) introducing packaging genes from a **retroviral vector** system into a cell line, which has substantially no endogenous proviruses that produce transcripts packageable by the **retroviral vector** system; and (b) selecting for cells that produce at least a ten-fold increase in viral packaging protein as compared to a standard mouse amphotropic packaging cell line, and that, upon introduction of a vector construct, produce at least a ten-fold

-more-

?

Display 6/9/13 (Item 1 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.
(c) 2003 Thomson Derwent & ISI. All rts. reserv.

increase in vector titer as compared to a standard mouse amphotropic

packaging cell line upon introduction of a vector construct, produce vector titers at least equivalent to those of a standard mouse amphotropic packaging cell line, and which produce vector particles capable of infecting human cells. In method (4), producing a transgenic packaging animal or insect comprises: (a) mating a transgenic animal or insect containing a vector construct coding for some, but not all, viral proteins necessary for packaging, with a transgenic animal or insect containing a vector construct coding for the remainder of the necessary viral proteins; and (b) isolating the progeny of the transgenic animals or insects. The method further comprises mating the progeny with a transgenic animal or insect containing a vector construct, to produce primary cells capable of producing high titer recombinant retrovirus. The method further includes infecting cells explanted from the progeny with a recombinant **retrovirus** containing a **vector** construct to produce primary cells capable of producing high titer recombinant retrovirus. In method (6),

-more-

?
Display 6/9/13 (Item 1 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.

producing a vector capable of infecting a cell type comprises: (a) continuously passaging a virus in cells of the selected cell type until the virus has genetically mutated and a predominant fast growing strain has evolved; (b) isolating the mutated and fast growing strain; (c) identifying and isolating the components of the mutated strain responsible for the preferential growth of the mutated virus; (d) inserting the identified and isolated components as substitutes for counterpart components in a producer cell based upon the virus prior to its continuous passage; and (e) culturing the producer cell to produce the vector. USE - The packaging cell lines are useful for producing recombinant **retroviruses** or **retroviral vector** particles. The packaging cell lines are useful as drug transport systems, particularly for delivering vector constructs to susceptible target cells, or for delivering or expressing proteins or therapeutic agents in target cells. ADVANTAGE - The non-mouse packaging cell line produces at least a ten-fold increase in viral packaging protein as compared to a standard mouse amphotropic packaging cell line. These are

-more-

? d s6/9/14-17
Display 6/9/14 (Item 1 from file: 370)
DIALOG(R)File 370:Science

00509660 (THIS IS THE FULLTEXT)
A Role for DNA-PK in Retroviral DNA Integration
Daniel, Rene; Katz, Richard A.; Skalka, Anna Marie<CRF RID="C1">
Institute for Cancer Research, Fox Chase Cancer Center, 7701 Burholme Avenue, Philadelphia, PA 19111, USA.
Science Vol. 284 5414 pp. 644
Publication Date: 4-23-1999 (990423) Publication Year: 1999
Document Type: Journal ISSN: 0036-8075
Language: English
Section Heading: REPORTS
Word Count: 2265

Abstract: Retroviral DNA integration is catalyzed by the viral protein integrase. Here, it is shown that DNA-dependent protein kinase (DNA-PK), a host cell protein, also participates in the reaction. DNA-PK-deficient murine scid cells infected with three different retroviruses showed a

-more-

?

Display 6/9/14 (Item 1 from file: 370)
DIALOG(R)File 370:Science
(c) 1999 AAAS. All rts. reserv.

substantial reduction in retroviral DNA integration and died by apoptosis. Scid cell killing was not observed after infection with an **integrase-defective** virus, suggesting that abortive integration is the trigger for death in these DNA repair-deficient cells. These results suggest that the initial events in retroviral integration are detected as DNA damage by the host cell and that completion of the integration process requires the DNA-PK-mediated repair pathway

Text: Integration is an essential step in retroviral replication (B1) . Processing (nicking) of the viral DNA 3 (prime) ends and joining of these ends to staggered phosphates in the host DNA are carried out by the viral integrase (IN) protein (B2) . The initial linkage between viral and host DNA is a gapped intermediate in which the viral DNA 5 (prime) ends are unjoined. The processing and joining steps in the integration reaction have been reconstituted in vitro, with purified retroviral integrases and model viral and host DNA substrates. Repair of the gaps in vivo results in a 4- to 6-base pair repeat of host DNA flanking each proviral end, but this

-more-

?

Display 6/9/14 (Item 1 from file: 370)
DIALOG(R)File 370:Science
(c) 1999 AAAS. All rts. reserv.

final step has not yet been reproduced in vitro. It has been reported that inhibition of a host DNA repair-related protein, poly(adenosine diphosphate-ribose) polymerase, blocks retroviral integration (B3) . Although it is generally assumed that host cell repair enzymes complete the integration reaction, the pathways responsible and the mechanism by which repair is accomplished have not been identified.

In mammalian cells, repair of double-stranded DNA breaks by nonhomologous end joining (NHEJ) is mediated by DNA-dependent protein kinase (DNA-PK). DNA-PK is composed of a DNA-binding Ku70/Ku86 heterodimer and a large catalytic subunit, DNA-PK.inf(cs) (B4) . DNA-PK also functions in V(D)J recombination, and the underlying genetic defect in the V(D)J recombination- deficient, severe combined immunodeficiency (scid) mouse (B5) is a truncation mutation of DNA-PK.inf(cs) (B6) . Thus, scid cell lines and primary cells are deficient in DNA-PK activity (B7) .

To investigate whether DNA-PK has a role in the repair process that completes retroviral integration, we infected scid cells with **retrovirus vectors**. scid pre-B cell lines S7, S29, and S33 and

-more-

?

Display 6/9/14 (Item 1 from file: 370)
DIALOG(R)File 370:Science
(c) 1999 AAAS. All rts. reserv.

a control, normal cell line, N2 (B8) , were first infected with an avian **retrovirus vector**, encoding an amphotropic envelope protein that allows infection of a wide variety of mammalian cells [RCASBP-M(4070A), hereafter abbreviated R/M] (B9) . The results (Fig. 1A) were striking; after infection with the vector at a multiplicity of ~2 infectious units (i.u.) per cell, viability (B10) of the scid cells dropped to 40 to 50%, whereas the viability of the N2 cells remained >90%. Cell death was dependent on the virus concentration, and it was first observed 12 hours after infection, indicating that an early event in the retroviral replication cycle was responsible.

To investigate this phenomenon further, we constructed matched derivatives of the original avian **retrovirus vector** (B11) . One

virus was integration-competent (IN.sup(+)); the other was integration-defective (IN.sup(-)), as it encoded a Asp.sup(64) --> Glu (D64E) substitution in the conserved D,D35,E motif, which makes up the catalytic center of avian sarcoma virus (ASV) integrase. All other early steps, including synthesis of viral DNA and its entry into the nucleus,

-more-

?
Display 6/9/14 (Item 1 from file: 370)
DIALOG(R)File 370:Science
(c) 1999 AAAS. All rts. reserv.
were normal in the IN.sup(-) mutant (B12) . Death of the scid cells occurred after infection with the IN.sup(+) virus, but not the IN.sup(-) virus (Fig. 1B), suggesting that this response was dependent on retroviral IN activity and was not merely an antiviral response induced by uptake of virus.

scid cells are highly sensitive to DNA damage, which appears to trigger apoptosis (B13) . Retroviral infection seemed to elicit a similar response. Application of the TdT-mediated deoxyuridine triphosphate nick end labeling (TUNEL) assay confirmed that infection of scid cells with the avian virus vector induced apoptosis in about 33 to 45% of infected cells at 12 and 16 hours after infection, respectively (Fig. 1C) (B14) . In contrast, no increase in apoptosis was detected after infection of the N2 cells. Similar results were obtained when apoptosis was evaluated by detection of cytoplasmic histone-associated DNA fragments and increase in caspase-3 activity, a marker for early events in apoptosis (B12) (B15) . Thus, we conclude that the retrovirus-infected scid cells die by apoptosis.

We considered the possibility that the scid cell lines may have

-more-

?
Display 6/9/14 (Item 1 from file: 370)
DIALOG(R)File 370:Science
(c) 1999 AAAS. All rts. reserv.
distinct phenotypes due to their lymphoblastoid lineage, immortalization, or passage history. Therefore, the efficiency of stable retrovirus integration was tested in scid mouse embryo fibroblasts (B16) with the IN.sup(+) vector that carries a neomycin resistance gene (neo.sup(r)) (B11) . The number of stable G418-resistant colonies was 10-fold lower in the infected scid fibroblasts than in matched normal primary mouse embryo fibroblasts (Table 1). We also tested the ability of the same vector to integrate in cell lines that are deficient in other components of the DNA-PK pathway, Ku, and the XRCC4 protein believed to be important for recruiting ligase IV (B17) . Similar reductions were observed in infected Ku86(-) and XRCC4(-) Chinese hamster ovary (CHO) cells when compared with control CHO cells (Table 1). Such reductions are expected if the majority of the scid, Ku(-), and XRCC4(-) cells die because they cannot repair the integration intermediate. Increased production of cytoplasmic histone-associated DNA fragments in the infected scid fibroblasts suggested that they also die by apoptosis (B12) . Thus, we conclude that scid fibroblasts and pre-B cells respond to retrovirus infection similarly and

-more-

?
Display 6/9/14 (Item 1 from file: 370)
DIALOG(R)File 370:Science
(c) 1999 AAAS. All rts. reserv.
that the efficiency of integration is reduced substantially in the absence of the three components of the DNA-PK pathway that are missing or deficient in the mutant cell lines (B17) .

We then examined whether another retrovirus could induce cell death in scid cells. For these experiments, we used a vesicular stomatitis virus G protein-pseudotyped human immunodeficiency virus type-1 (HIV-1) vector in

which the viral genes were replaced with a lacZ reporter gene under control of a cytomegalovirus promoter; (beta) -galactosidase ((beta) -Gal) was expressed efficiently only after vector integration (B18) . Infection with this vector also induced cell death in scid cells (Fig. 2A), and (beta) -Gal expression (B19) was reduced substantially in these cells (Fig. 2B). Thus, scid cells also appear to undergo apoptosis in the absence of repair of the HIV-1 DNA integration intermediate. Finally, similar scid cell killing was also observed after infection with Moloney murine leukemia virus (B12) .

To determine if DNA-PK activity increases as a consequence of retroviral integration, we studied HeLa cells, in which DNA-PK activity can

-more-

?

Display 6/9/14 (Item 1 from file: 370)
DIALOG(R)File 370:Science
(c) 1999 AAAS. All rts. reserv.

be detected readily upon stimulation by addition of damaged (sheared) DNA (B20) (Fig. 3A). HeLa cell cultures were infected with the IN.sup(+) or IN.sup(-) viruses, and samples were collected every 4 hours (Fig. 3B). Nuclear lysates were then tested for DNA-PK activity in the absence of added sheared DNA. A twofold to threefold increase in DNA-PK activity in IN.sup(+) virus-infected cells occurred 8 to 16 hours after infection (Fig. 3B), whereas a negligible difference in activity was seen in IN.sup(-)-infected cells. Thus, DNA-PK activity is stimulated by an early event in retroviral replication that depends on IN and appears to be sensed as DNA damage. It seems likely that the critical event is formation of the integration intermediate.

These results indicate a role for the DNA-PK-mediated repair pathway in retroviral integration, but the requirement is not absolute. Although the efficiency is greatly reduced, some stable integration is observed in Ku86(-), XRCC4(-), and DNA-PKcs(-) scid cells (Table 1). At least two interpretations of these results are possible; residual function of the DNA-PK pathway could account for the residual integration activity, or an

-more-

?

Display 6/9/14 (Item 1 from file: 370)
DIALOG(R)File 370:Science
(c) 1999 AAAS. All rts. reserv.

alternative pathway may compensate partially for defects in the DNA-PK pathway. We note that our results do not exclude a role for viral proteins in either the primary or compensatory pathways. Although we favor the hypothesis that another pathway or pathways may compensate for deficiencies in DNA-PK, we note that of the cell lines tested, only the XRCC4(-) line is genetically null (B17) .

In the initial steps of V(D)J recombination, signal sequences direct cleavage by RAG-1 and RAG-2 proteins. In vitro studies have revealed that the RAG proteins catalyze reactions that are mechanistically similar to those carried out by retroviral IN (B21) . In V(D)J recombination, DNA-PK is required for joining of the cleaved nonhomologous DNA coding ends and is thought to interact with RAG proteins (B22) . The data presented here indicate that cellular DNA-PK-dependent DNA repair is also required to complete retroviral DNA integration. These results and the observation that both RAG and IN reactions are stimulated by HMG DNA-binding proteins (B23) strengthen the case for evolutionary relatedness of V(D)J recombination and retroviral integration (B24) .

-more-

? d s6/9/15-17

Display 6/9/15 (Item 2 from file: 370)
DIALOG(R)File 370:Science
(c) 1999 AAAS. All rts. reserv.

00500268 (THIS IS THE FULLTEXT)
In Vivo Gene Delivery and Stable Transduction of Nondividing Cells by a **Lentiviral Vector**
Naldini, Luigi; Blomer, Ulrike; Gallay, Philippe; Ory, Daniel; Mulligan, Richard; Gage, Fred H.; Verma, Inder M.; Trono, Didier
L. Naldini, U. Blomer, P. Gallay, F. H. Gage, I. M. Verma, D. Trono, Salk Institute, 10010 North Torrey Pines Road, La Jolla, CA 92037, USA. ; D. Ory and R. Mulligan, Whitehead Institute for Biomedical Research, 9 Cambridge Center, Cambridge, MA 02142, USA.
Science Vol. 272 5259 pp. 263
Publication Date: 4-12-1996 (960412) Publication Year: 1996
Document Type: Journal ISSN: 0036-8075
Language: English
Section Heading: Reports
Word Count: 3384

-more-

?
Display 6/9/15 (Item 2 from file: 370)
DIALOG(R)File 370:Science

Abstract: A **retroviral vector** system based on the human immunodeficiency virus (HIV) was developed that, in contrast to a murine leukemia virus-based counterpart, transduced heterologous sequences into HeLa cells and rat fibroblasts blocked in the cell cycle, as well as into human primary macrophages. Additionally, the HIV vector could mediate stable in vivo gene transfer into terminally differentiated neurons. The ability of HIV-based viral vectors to deliver genes in vivo into nondividing cells could increase the applicability of **retroviral vectors** in human gene therapy

Text: Until now, gene therapy protocols have often relied on **vectors** derived from **retroviruses** such as murine leukemia virus (MLV) (B1) (B2) . These vectors are useful because the genes they transduce are integrated into the genome of the target cells, a desirable feature for long-term expression. However, these **retroviral vectors** can only transduce dividing cells, which limits their use for in vivo gene transfer in nonproliferating cells such as hepatocytes, myofibers,

-more-

?
Display 6/9/15 (Item 2 from file: 370)
DIALOG(R)File 370:Science

hematopoietic stem cells, and neurons (B3) (B4) . The optimal gene transfer system would include a **retroviral vector** based on a virus, such as HIV and other lentiviruses, that can integrate into the genome of nonproliferating cells. In vitro, HIV can infect primary cultures of monocyte-derived macrophages (B5) as well as cell cycle-arrested CD4.sup(+) HeLa or T lymphoid cells (B6) . Central to this ability are karyophilic determinants contained in two virion proteins, matrix (MA) and Vpr. These proteins interact with the nuclear import machinery and mediate the active transport of the HIV preintegration complex through the nucleopore (B7) (B8) (B9) .

A three-plasmid expression system was used to generate HIV-derived **retroviral vector** particles by transient transfection, as described for other vectors (B10) (Fig. 1). Plasmid pCMV (Delta) R9, the packaging construct, contains the human cytomegalovirus (hCMV) immediate early promoter, which drives the expression of all viral proteins required in trans. This plasmid is defective for the production of the viral envelope and the accessory protein Vpu. The packaging signal (PSI) and

-more-

?

Display 6/9/15 (Item 2 from file: 370)

DIALOG(R)File 370:Science

adjacent sequences were deleted from the 5 (prime) untranslated region, but the 5 (prime) splice donor site was preserved. A polyadenylation [poly(A)] site from the insulin gene was substituted for the 3 (prime) long terminal repeat (LTR) at the end of the nef reading frame (B11) . This design eliminated cis-acting sequences crucial for packaging, reverse transcription, and integration of transcripts derived from the packaging plasmid (B12) . To broaden the tropism of the vector, we used a second plasmid that encodes a heterologous envelope protein for pseudotyping the particles generated by pCMV (Delta) R9 (B13) . Two variants of this construct were used: One variant encodes the amphotropic envelope of MLV (Ampho), and the other encodes the G glycoprotein of vesicular stomatitis virus (VSV G) (B14) . The latter envelope offers the additional advantage of high stability, which allows for particle concentration by ultracentrifugation (B15) . The third plasmid, the transducing vector (pHR (prime)), contains cis-acting sequences of HIV required for packaging, reverse transcription, and integration, as well as unique restriction sites for the cloning of heterologous complementary DNAs (cDNAs). Nearly 350 base

-more-

?

Display 6/9/15 (Item 2 from file: 370)

DIALOG(R)File 370:Science

pairs of gag as well as env sequences encompassing the Rev response element (RRE) flanked by splice signals were included in the pHR (prime) vector (B16) . This design had a dual purpose: first, to increase packaging efficiency, as both gag and env RNA determinants have been demonstrated to enhance this process (B17) , and second, to allow the efficient transcription and cytoplasmic export of full-length vector transcripts only in the presence of the HIV Tat and Rev regulatory proteins, both of which are encoded by the packaging plasmid, pCMV (Delta) R9. In the absence of these transacting factors, the only detectable expression originated from the internal promoter in the vector (B18) . The Escherichia coli (beta) -galactosidase ((beta) -gal) or the firefly luciferase coding sequences were inserted into pHR (prime) downstream of the hCMV immediate early promoter to serve as reporter genes.

Replication-defective retroviral particles were generated by transient cotransfection of 293T human kidney cells with the three-plasmid combination (B19) . MLV-derived packaging and transducing vectors served as controls (B20) . Media from the various transfectants were first assayed

-more-

?

Display 6/9/15 (Item 2 from file: 370)

DIALOG(R)File 370:Science

for transduction frequency on growing 208F rat fibroblasts (B21) . HIV-based (beta) -gal vectors yielded titers of 0.8 (+/-1.7) x 10.sup(5) (n = 3) transducing units (TU) per milliliter with the MLV(Ampho) envelope and 4 (+/-1.5) x 10.sup(5) (n = 6) TU/ml with the VSV envelope. These titers are comparable with those obtained with MLV-based vectors produced by the same method-10.sup(5) TU/ml with its own envelope, and 5 x 10.sup(5) Tu/ml when pseudotyped with the VSV envelope-and significantly higher than those previously reported for other HIV-based vectors (B17) (B22) . Potentially contributing to this increased efficiency is the incorporation of accessory HIV-1 genes into the packaging construct, including nef that markedly enhances virion infectivity (B23) .

The HIV-derived vector system used here is devoid of helper virus per

se. Furthermore, the use of a three-plasmid combination and of a heterologous envelope, as well as the removal of multiple cis-acting sequences from the packaging vector, makes it unlikely that a replication-competent recombinant would be generated. The potential transfer of packaging functions from producer to target cells was assayed

-more-

?
Display 6/9/15 (Item 2 from file: 370)
DIALOG(R)File 370:Science

by testing for the production of the tat and gag gene products in vector-transduced cells. Neither protein was detected, which, considering the sensitivity of the assays we used (B24) , implied that the transfer of packaging functions was at least three orders of magnitude less efficient than that of vector sequences. Furthermore, conditioned medium from serially passaged transduced cells did not transfer the reporter gene to naieve cells (B24) .

HIV-and MLV-derived vectors were compared for their ability to transduce cells blocked at various stages of the cell cycle. HeLa cells were growth-arrested at the G.inf(1)-S boundary or at the G.inf(2) phase of the cycle by aphidicolin treatment or gamma irradiation, respectively (B25) . The arrested state of the cells at the time of infection was verified by propidium iodide staining of the DNA and by flow cytometry (B18) . An HIV-based **retroviral vector** expressing (beta) -gal was as efficient at transducing G.inf(1)-S- and G.inf(2)-arrested as proliferating HeLa cells, whereas its MLV counterpart was only 5 to 8% as effective (Table 1). The wider variability observed in the transduction of HeLa

-more-

?
Display 6/9/15 (Item 2 from file: 370)
DIALOG(R)File 370:Science

cells arrested by gamma irradiation was perhaps due to the cytotoxicity of the treatment.

To test whether the HIV-based vector integrates in the host cell genome, we used packaging constructs carrying **mutations** that inactivate **integrase**. HIV-1 mutants in which the expression of integrase is abrogated by the introduction of a stop codon at its 5 (prime) end do not reverse transcribe their genome efficiently (B26) . When this mutation was introduced into the packaging construct, it completely prevented transduction by the resulting vector particles. Furthermore, whereas a (beta) -gal vector made with the wild-type packaging construct had a transduction efficiency of 940 TU per nanogram of p24 in growing or G.inf(1)-S-arrested cells, a single amino acid change [from aspartic acid to valine at position 64 (D64V)] in the HIV-1 integrase sequence, previously demonstrated to severely decrease the activity of this enzyme but not to affect any other step of infection (B27) , reduced the efficiency to 54 and 130 TU per nanogram of p24 in growing and G1-S-arrested cells, respectively (B28) . Efficient gene transfer in both

-more-

?
Display 6/9/15 (Item 2 from file: 370)
DIALOG(R)File 370:Science

settings was thus dependent on reverse transcription as well as integration. Taken together, these results indicate that the unique features of HIV can be transferred to a replication-defective **retroviral vector**, allowing transduction of nonproliferating cells.

To test the transduction of cells arrested in G.inf(0), we grew

cultures of rat 208F fibroblasts to confluence and then maintained them in G.inf(0) by density-dependent inhibition of growth in the presence of dexamethasone (B3) . The HIV-based vector was significantly more efficient than its MLV equivalent. However, its transduction rate decreased as a function of time between growth arrest and infection (Table 1). Cells growth-arrested for 4 days were transduced at levels that were 45% of those observed in dividing cells. However, in cells that had been maintained in G.inf(0) for 15 days, the relative transduction decreased to 17%. The MLV-based vector was significantly more affected by the growth arrest. In its case, the residual transducing activity reflected the fraction of cells still undergoing division, as assessed by propidium iodide staining of the

-more-

?
Display 6/9/15 (Item 2 from file: 370)
DIALOG(R)File 370:Science
(c) 1999 AAAS. All rts. reserv.
cell DNA followed by flow cytometry (B29) . Whereas vector particles entered G.inf(0)-arrested and dividing cells with comparable efficiencies (B30) , they were significantly defective for reverse transcription in G.inf(0) cells (Fig. 2), which resembles a phenomenon observed in HIV-infected quiescent T lymphocytes (B31) . Nevertheless, a stable transduction intermediate must have been established, because replating and proliferation of G.inf(0) cells up to 8 days after infection revealed titers as high as 50% of those obtained in dividing cells (Table 1). In contrast, inducing cell division even 1 day after inoculation did not rescue the MLV-derived vector. The generation of a stable infection intermediate by the HIV-based vector offers an advantage for delivering genes into targets such as hematopoietic stem cells. Indeed, it may alleviate the need for inducing the proliferation of these cells ex vivo, a manipulation that can affect their pluripotentiality.

The decreased transduction efficiency of the HIV vector in G.inf(0)-arrested fibroblasts may partly reflect suboptimal concentrations of intracellular deoxynucleotides (B32) . Whether a similar limitation

-more-

?
Display 6/9/15 (Item 2 from file: 370)
DIALOG(R)File 370:Science
(c) 1999 AAAS. All rts. reserv.
would preclude gene transfer into terminally differentiated primary cells could not be inferred from these observations and was therefore assessed directly. The HIV-based luciferase vector, pseudotyped with the VSV G protein, was tested for its ability to transduce human monocyte-derived primary macrophages (B33) . Significant levels of luciferase activity were detected in an envelope-dependent manner (Table 2). In contrast, only background levels of luciferase activity were measured in macrophages inoculated with a comparable VSV G-pseudotyped MLV-based vector (B34) . To rule out that the HIV vector was infecting a small proportion of macrophages that were proliferating, we generated mutant packaging constructs where Vpr and the nuclear localization signal (NLS) present in the MA protein were inactivated (B35) . At least one of these two elements is essential for viral infection in macrophages, because they mediate nuclear import of the HIV preintegration complex (B7) (B8) (B9) . A vector assembled from a mutant packaging construct in which both Vpr and the MA NLS are inactivated was severely reduced in its ability to transduce macrophages (Table 2). Similarly, NLS peptide treatment prevented

-more-

? d s6/9/16-17
Display 6/9/16 (Item 1 from file: 35)
DIALOG(R)File 35:Dissertation Abs Online

01750361 ORDER NO: AADAA-I9973164
Incorporation of integrase-LexA fusion proteins into HIV-1 and analysis of resulting virions, a strategy for achieving site-directed integration in vivo (Immune deficiency)
Author: Holmes, Michelle Lynne
Degree: Ph.D.
Year: 2000
Corporate Source/Institution: University of California, Los Angeles (0031)
Chair: Samson A. Chow
Source: VOLUME 61/06-B OF DISSERTATION ABSTRACTS INTERNATIONAL. PAGE 2855. 193 PAGES
Descriptors: HEALTH SCIENCES, PHARMACOLOGY ; BIOLOGY, MICROBIOLOGY ; BIOLOGY, MOLECULAR ; BIOLOGY, CELL
Descriptor Codes: 0419; 0410; 0307; 0379
ISBN: 0-599-78745-7

-more-

?
Display 6/9/16 (Item 1 from file: 35)

Retroviruses are leading candidates in the development of gene therapy vectors. This is due, in part, to their ability to stably and precisely introduce a DNA copy of their genome into the chromosomes of an infected cell. A potential pitfall also exists in this reaction because integration occurs nonspecifically with regard to the chromosomal DNA. <italic>In vitro </italic>, purified fusion proteins made up of a retroviral integrase and a sequence-specific DNA-binding protein, such as the LexA repressor of <italic> E. coli</italic>, are able to direct integration toward specific sites. To determine whether these proteins can be incorporated into HIV-1 and whether the resulting virions are infectious, an <italic>in trans</italic> approach was used to deliver various integrase-LexA proteins to a virus encoding a catalytically inactive integrase gene. Integrase-LexA, integrase-LexA DNA-binding domain and N- and C-terminal integrase-LexA were fused to the viral accessory protein, Vpr. Coexpression of the Vpr-fusion proteins with an **integrase-defective** HIV-1 molecular clone resulted in virus production from packaging cells and

-more-

?
Display 6/9/16 (Item 1 from file: 35)

efficient incorporation of the fusion proteins into virus particles. Cellular assays, measuring virus infectivity by stable expression of a reporter gene, revealed that each of the integrase-LexA fusion proteins was able to restore integration, and infectivity, to the integrase-deficient viral clone. To examine the mechanism by which the integrase-LexA proteins mediated integration, the virus-host DNA junctions of the integrated viral genomes were sequenced. In proviruses formed by the **integrase-mutated** virus containing the **integrase**-LexA protein, the characteristic hallmarks of integration were present, implying that the fusion proteins mediated insertion of the viral DNA like wild-type **integrase**. In addition, the aspartate 64 **mutation** was maintained, suggesting that a reversion of the integrase gene to wild type was not responsible for the integration events. Mixed multimers between the catalytically inactive integrase and the fusion proteins in the viruses were also determined to be formed, using complementation experiments. These results demonstrate the feasibility producing site-directed **retroviral vectors** using the integrase-fusion protein approach.

-more-

?
Display 6/9/16 (Item 1 from file: 35)
DIALOG(R)File 35:Dissertation Abs Online

The proteins can be efficiently incorporated into HIV-1, and mediate integration in a manner consistent with the wild-type virus.

- end of record -

?
Display 6/9/17 (Item 1 from file: 50)
DIALOG(R)File 50:CAB Abstracts

03456872 CAB Accession Number: 970107120
Avian retrovirus U3 and U5 DNA inverted repeats. Role of nonsymmetrical nucleotides in promoting full-site integration by purified virion and bacterial recombinant integrases.
Vora, A. C.; Chiu, R.; McCord, M.; Goodarzi, G.; Stahl, S. J.; Mueser, T. C.; Hyde, C. C.; Grandgenett, D. P.
St. Louis University Health Sciences Center, Institute for Molecular Virology, St. Louis, Missouri 63110, USA.
Journal of Biological Chemistry vol. 272 (38): p.23938-23945
Publication Year: 1997
ISSN: 0021-9258 --
Language: English
Document Type: Journal article
The U3 and U5 termini of linear retrovirus DNA contain imperfect inverted repeats that are necessary for the concerted insertion of the termini into the host chromosome by viral integrase. Avian myeloblastosis

-more-

?
Display 6/9/17 (Item 1 from file: 50)
DIALOG(R)File 50:CAB Abstracts

virus integrase can efficiently insert the termini of retrovirus-like DNA donor substrates (480 bp) by a concerted mechanism (full-site reaction) into circular target DNA in vitro. The specific activities of virion-derived avian myeloblastosis virus integrase and bacterial recombinant Rous sarcoma virus (Prague A strain) integrase (less than or equal to 50 nM) appear similar upon catalyzing the full-site reaction with 3'-OH recessed wild type or mutant donor substrates. The role of the 3 non-symmetrical nucleotides located at the 5th, 8th, and 12th positions in the U3 and U5 15-bp inverted repeats was examined for their ability to modify the full-site and simultaneously, the half-site strand transfer reactions. It is suggested that the nucleotide at the 5th position is responsible for the 3-5-fold preference for wild type U3 ends over wild type U5 ends by **integrase** for concerted integration. Additional **mutations** at the 5th or 6th position, or both, of U3 or U5 termini significantly increased (approx equal to 3 fold) the full-site reactions of mutant donors over wild type donors. 30 ref.

-more-

? e au=engelman alan

Ref	Items	Index-term
E1	145	AU=ENGELMAN A
E2	60	AU=ENGELMAN A.
E3	66	*AU=ENGELMAN ALAN
E4	2	AU=ENGELMAN ARNO
E5	8	AU=ENGELMAN B

```
E6        2  AU=ENGELMAN B E
E7        3  AU=ENGELMAN B P
E8        1  AU=ENGELMAN B.
E9        1  AU=ENGELMAN B.E.
E10       1  AU=ENGELMAN BE
E11       1  AU=ENGELMAN BP
E12      10  AU=ENGELMAN C

          Enter P or PAGE for more
? e au=engelman alan

Ref   Items  Index-term
E1      145  AU=ENGELMAN A
E2       60  AU=ENGELMAN A.
E3       66 *AU=ENGELMAN ALAN
E4        2  AU=ENGELMAN ARNO
E5        8  AU=ENGELMAN B
E6        2  AU=ENGELMAN B E
E7        3  AU=ENGELMAN B P
E8        1  AU=ENGELMAN B.
E9        1  AU=ENGELMAN B.E.
E10       1  AU=ENGELMAN BE
E11       1  AU=ENGELMAN BP
E12      10  AU=ENGELMAN C

          Enter P or PAGE for more
? e au=engelman, alan

Ref   Items  Index-term
E1       30  AU=ENGELMAN, A.
E2        1  AU=ENGELMAN, A. E
E3       59 *AU=ENGELMAN, ALAN
E4        2  AU=ENGELMAN, ALAN N
E5        2  AU=ENGELMAN, B.
E6        1  AU=ENGELMAN, B. E.
E7        2  AU=ENGELMAN, B. P.
E8        1  AU=ENGELMAN, BENJAMIN
E9        2  AU=ENGELMAN, BILL R.
E10       2  AU=ENGELMAN, BOB
E11       1  AU=ENGELMAN, BRUCE E.
E12       5  AU=ENGELMAN, C.

          Enter P or PAGE for more
? e au=hofmann wolfgang

Ref   Items  Index-term
E1       64  AU=HOFMANN WOLF-K
E2       65  AU=HOFMANN WOLF-KARSTEN
E3       79 *AU=HOFMANN WOLFGANG
E4        5  AU=HOFMANN WOLFGANG E
E5        7  AU=HOFMANN WOLFGANG J
E6        6  AU=HOFMANN WP
E7        1  AU=HOFMANN WT
E8       22  AU=HOFMANN WW
E9       42  AU=HOFMANN Y
E10       4  AU=HOFMANN Y.
E11       9  AU=HOFMANN YVONNE
E12       1  AU=HOFMANN- CARRETERO, S.

          Enter P or PAGE for more
? s e3 and integrase
            79  AU=HOFMANN WOLFGANG
         17641  INTEGRASE
```

S7 0 AU='HOFMANN WOLFGANG' AND INTEGRASE

?